PRELIMINARY AMENDMENT
DIV of USSN 09/000,442

The above amendments are consistent with the amendments made to Parent Application Serial No. 09/000,442.

Hence, the amendments to the specification and claims do not constitute new matter, and thus entry is respectfully requested.

The Examiner is invited to contact the undersigned at his Washington telephone number on any questions which might arise.

Respectfully submitted,

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A P P E N D I X

Marked-Up Version of Changes

IN THE SPECIFICATION:

Page 1, before line 3, the following paragraph is being inserted

-- This is a Divisional of Application Serial No. 09/000,442, filed May 19, 1998 (now allowed), which is a 371 of PCT/AU96/00472, filed July 26, 1996. The disclosures of each of which are incorporated herein by reference. --

Page 10, lines 6-14, are changed as follows:

"**Figure** 5A **[5]** provides a graph showing transient expression of PGKlacOcat in CHO/M(1)2lacIN + / - metal and + / - IPTG. CHO/M(1)2lacIN cells were transiently transfected with PGKlacOcat, the cells were incubated for 48 hours post transfection in medium + / - metal and + / - IPTG. Levels of chloramphenicol transferase (cat) protein were measured in cell extracts. Levels of cat were expressed relative to uninduced levels set at 100%. Repression of >95% of the PKLlacOcat gene were observed in the presence of metal, and IPTG led to a derepression indicating that the observed repression was specific to the *lac* repressor."

Page 10, lines 16-25, are changed as follows:

"**Figure** 5B **[5A]** shows, diagrammatically, the incorporation of *lacO* sequences in CMVTf to generate CMVlacOTF. The 18 base ideal *lacO* sequence was used to replace 18 bases in the CMV promoter between the TATAA (SEQ ID NO:4) box and transcription start point (tsp), and a dimer of that sequence was inserted into the restriction site *Pme* I between the tsp and the ATG start codon. The replacement was achieved by incorporating the *lacO* sequence in an oligonucleotide then used as a primer in the Polymerase Chain

Reaction (PCR). The insertion of the *lacO* dimer was done by ligation of a restriction enzyme fragment from the plasmid pOP (Hannan, G., et al 1994) into the *Pme* I site of PCMVTf."

Page 10, lines 27-30, and Page 11, lines 1-7, are changed as follows:

"**Figure 5C [5B]** provides a graph demonstrating the stable expression of CMVlacOTf in CHO/M(1)2lacIN. The plasmid pCMVlacOTf was transfected with CHO cells and stable transformants selected (labelled CHO/M(1)2lacIN cells). Cells from 2 clonal lines were grown for 12 hours in medium +/-metal and +/-IPTG, fresh medium added and the conditioned medium collected after another 24 hours. Tf levels were estimated (visually) on a Western blot and are expressed relative to levels in the absence of metal and IPTG which was set at 100%. Metal induced repression levels of >90% was observed in both clones and IPTG relieved most of that repression indicating its specificity. Levels of IPTG used (20mM) may have been sub-optimal thus explaining the incomplete derepression observed."

Page 15, lines 8-20, are changed as follows:

"**Example 1: The expression of transferrin or IGF-1 in CHO cells**

Work over the last few years has been conducted in order to gain an understanding of the growth requirements of CHO-K1 in serum free medium (Crowley, J., 1989, Gray, P.P. et al., 1990 and Bridges, M., PhD Thesis). Long term growth from liquid nitrogen to large scale may be obtained with insulin or insulin-like growth factor (IGF), transferrin and fibronectin or laminin as the only exogenous proteins. Selenium also needs to be added as a trace element. A defined serum-free (SF) medium was developed referred to as UNSWSF+ITS. Slight changes in growth characteristics of the CHO K1 cell line have occurred with different samples of CCL61

obtained over the years from American Type Culture Collection <u>(ATCC)</u> [(ATEC)]. The current CHO K1 CCL61 stock obtained from ATCC in 1994 grows with a doubling time of around 17 hours in UNSWSF+ITS medium."

Page 15, lines 23-28, are changed as follows:

"The coding sequences for the IGF1 and transferring (Tf) genes, including the sequences for protein secretion, were isolated from a commercial human liver cDNA library (Clontech) using Polymerase Chain Reaction. Sequences 5' of the AUG start codon were modified to include an optimal translation initiation site (ACCATGA <u>(SEQ ID NO:1)</u> replacing AAGATGA <u>(SEQ ID NO:2)</u>, Kozak, M., 1986)."

Page 18, lines 7-16, are changed as follows:

"Figure 4A shows that the presence of transferrin in defined medium, helped the long term growth and viability of CHO-K1 cells in culture. Figure [5] <u>4B</u> shows the final cell numbers and percentage viability of CHOSVLTf cells maintained from greater than 10 days in IS medium. It can be seen from Figure 4B that while the final cell densities for the three cell lines were similar, CHO-K1 growing on UNSWSF-ITS medium had a viability of 60%, similar to the CHOSVLTf cells expressing transferrin, while the viability of the CHO-K1 cells growing on the UNSWSF+IS medium was only 20%. This data shows the importance of having the CHO cells secreting transferrin, particularly for long term stable growth and viability."

Page 19, lines 3-10, are changed as follows:

"CHO cells were transfected using standard techniques with [M(1)2lacI^{N}] <u>M(1)2lacIN</u> and pSV2Neo. Cells were treated with 400 μg/ml of G418 for 2 weeks and clonal cell lines resistant to G418 were selected. Clonal lines (designated CHO/R(5)4 and

CHO/R(10)3) were obtained that produced low basal and high metal induced levels of repressor, as detected by Western blot. The repressor was shown to be biologically active by its ability to repress the lacO-containing plasmid PGKlacOcat (Hannan, G., et al., 1994) transiently introduced into the cells [(Fig. 5)] (Fig. 5A)."

Page 19, lines 13-20, are changed as follows:

"An ideal *lac* operator sequence (ATTGTGAGCGCTCACAAT (SEQ ID NO:3)) based on the bacterial operator and rules for acceptable sites of insertion within a given promoter have been described (Hu & Davidson, 1987). The bacterial *lac* repressor has a high association constant for the ideal *lac* operator sequence which is a rare sequence with only three copies found in various mammalian genomes (Simons et al., 1984), thus offering good specificity of regulation of the target gene and minimal effect on the host genes (Simons et al., 1984)."

Page 19, lines 21-28, are changed as follows:

"*Lac* operator sequences were inserted into the CMVTf gene to allow for repression by the *lac* repressor. One *lac* operator sequence [(Fig. 5A)] (Fig. 5B) replaced promoter wild-type sequences between the TATAA (SEQ ID NO:4) box and the transcription start point and the other two were inserted between the transcription start point and the AUG start codon. Stable expression of this new construct (CMVlacOTf) in CHO cells already containing a stable inducible *lac* repressor gene (see example 3) was significantly shut down when repressor protein was present (i.e., the following metal induction) [(Fig 5B)] (Fig 5C)."

IN THE CLAIMS:

Claims 1-6 and 17-21 are being cancelled.

The claims are amended as follows:

Claim 7. (Amended) A method for the regulated growth of a mammalian host cell in a culture medium, comprising the step of:

culturing said mammalian host cell in said culture medium, wherein said host cell includes:

(i) at least one introduced DNA sequence encoding a protein, polypeptide and/or peptide factor(s) required for growth of the host cell in said culture medium [expressibly] operably linked to a promoter sequence, the expression of which is regulated by a repressor binding region; and

(ii) at least one introduced DNA sequence encoding a repressor molecule which binds to the repressor binding region, [expressibly] operably linked to an inducible promoter sequence.

Claim 11. (Amended) A method according to [any one of the preceding claims] Claim 10, wherein the DNA sequence(s) encoding the protein, polypeptide and/or peptide growth factor(s) encodes a growth factor(s) selected from the group consisting of insulin, modified insulins, insulin-like growth factors, cytokines, mitogenic proteases and mixtures thereof.

Claim 14. (Amended) A method according to [any one of the preceding claims] Claim 7, wherein the culture medium is protein/serum-free medium.

Claim 15. (Amended) A method according to [any one of the preceding claims] Claim 7, wherein the mammalian host cell is a Chinese hamster ovary cell.

Claim 16. (Amended) A method according to [any one of the preceding claims] Claim 15, wherein the mammalian host cell is a CHO-K1 cell.

Claim 22. (Amended) A host cell including:

(i) at least one introduced DNA sequence encoding a protein, polypeptide and/or peptide factor(s) required for growth of the host cell in a protein/serum-free culture medium [expressibly] operably linked to a promoter sequence, the expression of which is regulated by a repressor binding region; and

(ii) at least one introduced DNA sequence encoding a repressor molecule which binds to the repressor binding region, [expressibly] operably linked to an inducible promoter sequence.

Claim 26. (Amended) A host cell according to [any one of claims 17 to 25] Claim 22, wherein the DNA sequence(s) encoding the protein, polypeptide and/or peptide growth factor(s) encodes a growth factor(s) selected from the group consisting of insulin, modified insulins, insulin-like growth factors, cytokines, mitogenic proteases and mixtures thereof.

Claim 29. (Amended) A host cell according to [any one of claims 17 to 28] Claim 22, wherein the mammalian host cell is a Chinese hamster ovary cell.

Claim 30. (Amended) A host cell according to [any one of claims 17 to 29] Claim 29, wherein the mammalian host cell is a CHO-K1 cell.